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Trimodal adult emergence in summer generations of the rose sawfly *Arge nigrinodosa* (Hymenoptera, Argidae)

Maya Kawasaki[1,†], Mariko Fujita[1,‡], Atsushi Sakurai[1,§], Kaoru Maeto[1,§]

1 *Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe 657-8501, Japan*

Corresponding author: *Kaoru Maeto* (maeto@kobe-u.ac.jp)

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Abstract

We explored the variable adult emergence in summer generations of a multivoltine sawfly [*Arge nigrinodosa* (Argidae)], larvae of which feed gregariously on the foliage of *Rosa* spp. (Rosaceae), and its ecological significance. The sawfly showed a trimodal adult emergence under long-day conditions in the laboratory. Following the first and largest cluster of emergence, a small tail of slightly delayed emergence was observed, which most likely was heritable. The third cluster of emergence after nonheritable partial diapause in prepupae seemed to match the synchronous emergence of a portion of adults in September under field conditions, probably as a risk-spreading (i.e., bet-hedging) strategy to cope with food shortage during unpredictable periods of drought in summer.

Keywords

Bet-hedging, partial summer diapause, polymodal emergence, risk-spreading, stochastic polyphenism, voltinism

Introduction

Seasonal life cycles are often variable within a population of herbivores (e.g., Danks 1994), but little is known about the life-history variability of sawflies with the exception of some conifer-feeding species (e.g., Knerer 1993; Maeto and Ozaki 1993). Within the genus *Arge* (Hymenoptera, Argidae), whose larvae feed on various dicotyledon plants (Smith 1989), voltinism is often confusing due to apparently irregular or

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polymodal adult emergence, as indicated by field and laboratory observations (Regas-Williams and Habeck 1979; Adachi 1983a; Shinohara et al. 2007, 2008; Shinohara and Hara 2008, 2009; Hara 2010). For example, *Arge suzukii* (Matsumura), which feeds on *Abelia* spp. (Linnaeaceae), exhibits a multivoltine life cycle (Okutani 1956), whereas its actual life history appears quite complicated given that adult emergence is polymodal and irregular under constant rearing conditions (Shinohara and Hara 2008). Additionally, *Arge pullata* (Zaddach) feeding on *Betula* spp. (Betulaceae) probably has two generations a year, but often with adult emergences deviating from this pattern (Hara and Shinohara 2008). Such occurrences led Shinohara and Hara (2008) to hypothesize that the concept of voltinism does not make sense in many *Arge* sawflies. More detailed and experimental studies are necessary to understand the nature and ecological implications of variable life histories in *Arge*.

Arge nigrinodosa (Motschulsky) is a common sawfly that feeds gregariously on the foliage of *Rosa* spp. (Rosaceae) in Japan and the Russian Far East (Tokunaga et al. 1951; Okutani 1967; Shinohara 2005). The females lay an average of ca 40 eggs in several egg masses in young and soft rose shoots. The hatched larvae form compact aggregations on a few leaflets near the tips of the shoots and often cause serious damage to wild and cultivated roses (Tokunaga and Tsujita 1951; Adachi 1981, 1983a). One generation requires approximately 45 days at 25 °C (Adachi 1981). Adult sawflies appear twice in late June and late August in northern Japan (Hokkaido) (Hara 2010), and continuously from late April to October in the lowlands of southern Japan (western Honshu) (Adachi 1983a; Yoshida 2006). Eggs deposited on wild roses can almost always be observed from May to October in western Honshu, but the egg density fluctuates markedly (Fig. 1 in Adachi 1983a), making an assessment of the number of generations per year difficult. Moreover, larval abundance appears to increase in the fall following a summer decline (Osaka Plant Protection Association 2003). The puzzling seasonal patterns in this multivoltine sawfly may be due to partial summer diapause before adult emergence, which is the case for the noctuid *Mamestra brassica* (Masaki and Sakai 1965) as well as some other insects (Masaki 1980), in addition to variable adult emergence of the overwintered generation in spring (Adachi 1983a).

To explore the variable life cycle of *A. nigrinodosa*, we examined the temporal variability of adult emergence, its heritability, and the prepupal development of the sawfly under constant conditions in the laboratory. The ecological significance of variable adult emergence is discussed with reference to changes in the abundance of larval food resources detected in the field.

Materials and methods

Locality and weather conditions

Arge nigrinodosa eggs and larvae were collected at the campus of Kobe University (34°43.7'N, 135°14.1'E; altitude ca. 200 m), Kobe City, Hyogo Prefecture, western Honshu, Japan, and a field census of wild roses [*Rosa multiflora* (Rosaceae)] was con-

ducted in the same place. Insects were reared in the laboratory at Kobe University or under outdoor field conditions at Karatodai (34°47.4'N, 135°12.8'E; altitude ca. 350 m), Kobe City. The monthly mean natural day length including one hour of twilight in this area is about 14.1 h in April, 15.0 h in May, 15.5 h in June, 15.3 h in July, 14.6 h in August, 13.4 h in September, and 12.5 h in October.

Figure 1 shows the monthly mean air temperatures and monthly precipitation from 1981 to 2010 at the Kobe Meteorological Station (34°41.8'N, 135°12.7'E) (Japan Meteorological Agency), indicating that August is the hottest and driest month on average. In August, the monthly precipitation is often less than 25 mm (Fig. 2).

Variability of adult emergence under long-day conditions and its heritability

To record the number of days from cocoon spinning to adult emergence, eggs and young larvae collected in May and June 2006 were reared at 20°C with a 16L–8D photoperiod in transparent plastic boxes (14 cm diameter, 7 cm depth) containing fresh leaves of *R. multiflora*, which were renewed every 5 - 10 days. The emerged virgin female adults were placed individually in the same type of transparent plastic box, each containing a young shoot of *R. multiflora* provided for oviposition, and kept at 25°C with a 16L–8D photoperiod. To record the number of days from cocoon spinning to

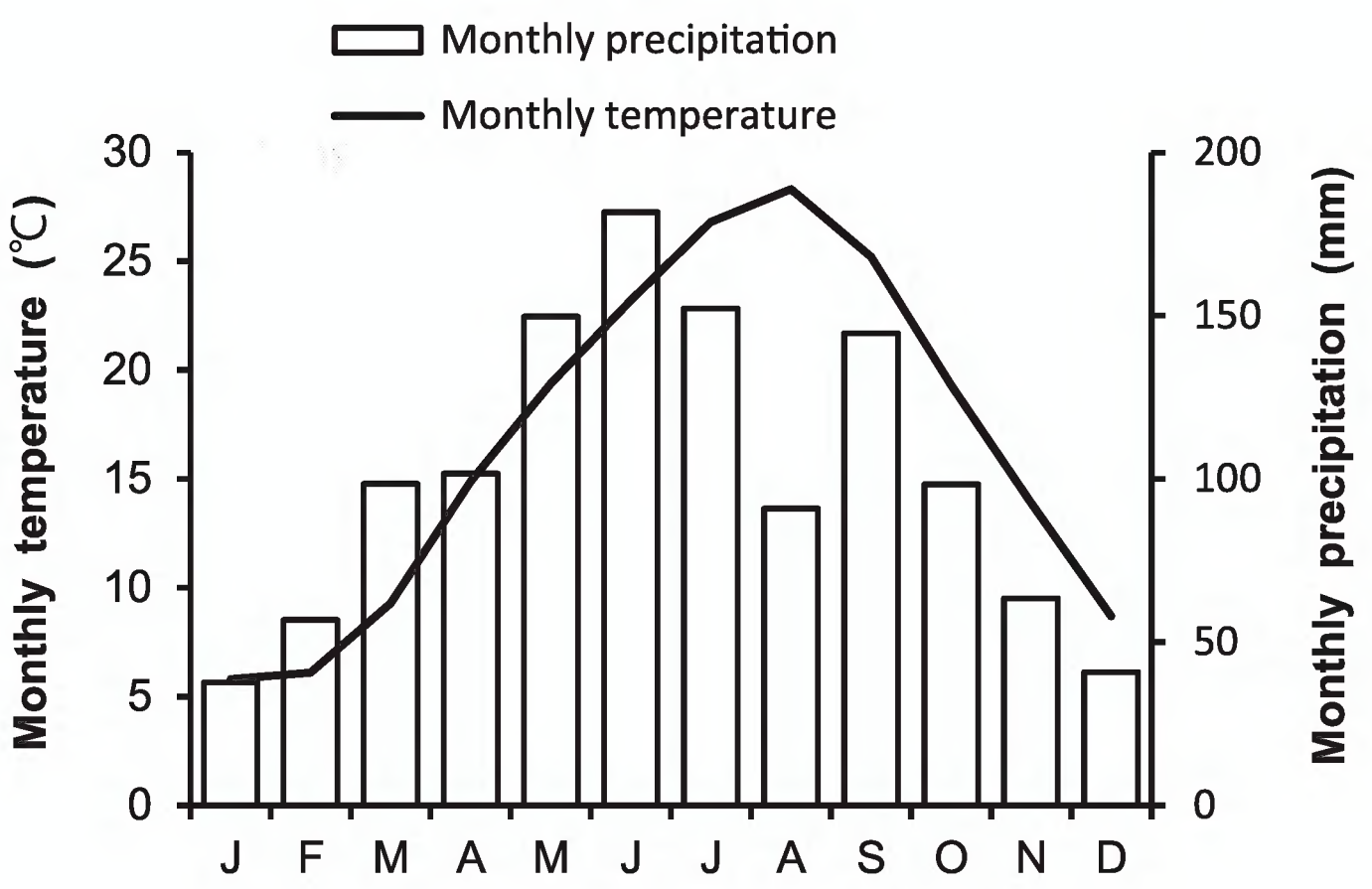

Figure 1. Monthly mean air temperatures and monthly precipitation from 1981 to 2010 at the Kobe Meteorological Station.

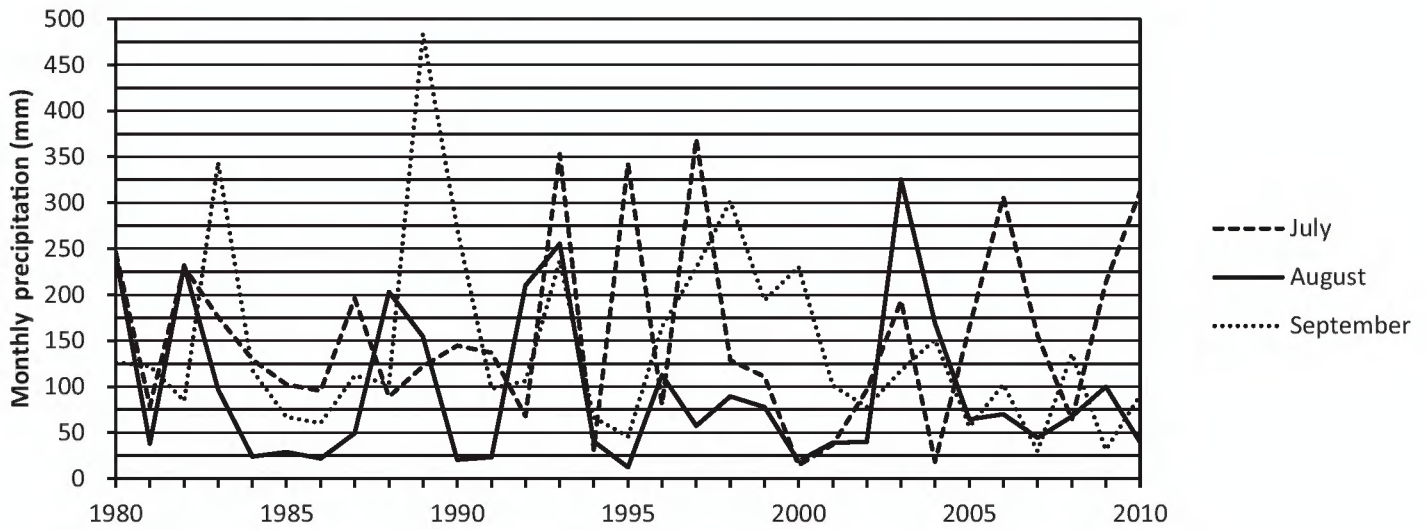

Figure 2. Annual changes in the monthly precipitation in July, August, and September from 1980 to 2010 at the Kobe Meteorological Station.

adult emergence, larvae hatched from deposited eggs (all haploid males) were reared at 20°C with a 16L–8D photoperiod.

Adult emergence under field conditions in summer generations

Eggs or larvae were collected from the foliage of *R. multiflora* on 24 May and 4 July 2007. They were reared outdoors, under shaded conditions, in transparent plastic boxes (14 cm diameter, 7 cm depth) containing fresh leaves of *R. multiflora*, which were renewed whenever wilted. Cocoons spun by larvae were moved to individual small transparent glass tubes (1 cm diameter, 4 cm length), with the opening covered with aluminum foil. The glass tubes were grouped together in transparent plastic boxes (14 cm diameter, 7 cm depth). The dates of cocoon spinning, adult emergence, and sex of the adults were recorded.

Shoot phenology of wild roses.

In early April 2007, 40 stem shoots of the current year's growth of *R. multiflora* were chosen for successive measurements. The shoot lengths were measured and the number of lateral shoots emerging from the stems was recorded every two weeks from late April to early October.

Prepupal development in cocoons

We observed the formation of pupal compound eyes through the translucent prepupal head capsules. The outline of the pupal eye (shaped like an eyebrow) increased in length during the transition from eonymph to pronymph (cf. Hamel et al. 1998).

Eggs collected on 17 May 2008 were reared under field conditions as described above. Using forceps we dissected eight cocoons from which adults had not emerged within 40 days after cocoon spinning, and we measured the length of pupal eye outlines of prepupae every week until pupation with a digital microscope (VH-8000, Keyence, Osaka, Japan).

Eggs collected in June 2008 were reared at 20°C with a long-day (16L–8D) or short-day (13L–11D) photoperiod. Under the long-day photoperiod, most adults emerged by 20 days after cocoon spinning while a portion of individuals entered what was presumably summer diapause. We dissected cocoons immediately after spinning and those remaining 25 days after spinning to measure the length of pupal eye outlines until pupation. Under the short-day photoperiod, all individuals entered what was presumably winter diapause; we dissected cocoons to measure the length of pupal eye outlines from 39 to 61 days after cocoon spinning.

Results

Variability of adult emergence under a long-day condition and its heritability

Under a constant temperature of 20°C with a photoperiod of 16L–8D, adult emergence exhibited a trimodal pattern (Fig. 3). The first and largest cluster of emergence occurred 13–24 (males) and 13–28 (females) days after cocoon spinning, while the second emergence occurred 25–36 (males) and 31–40 (females) days after cocoon spinning. The first and second clusters were distinctly separated for females (Fig. 3a), whereas they were rather continuous for males (Fig. 3b). The third cluster of emergence occurred 45–64 (males) and 49–62 (females) days after cocoon spinning. The first, second, and third clusters of emergence included approximately 2/3, 1/6, and 1/6 of adults, respectively, for both sexes (Fig. 3).

Figure 4 shows adult emergence in the sons of mothers in the first, second, and third clusters of emergence. The sons of all three types of mothers exhibited the three clusters of emergence, except that no second cluster of emergence occurred for the sons of mothers in the first cluster (Fig. 4a). Assuming that the range of an emergence period for the first, second, and third clusters of sons is respectively 12–26 days, 27–40 days, and 41–65 days after cocoon spinning (Fig. 5), the proportion of the second cluster of sons to all sons was significantly different among the clusters of mothers (Fisher's exact probability test, $p < 0.001$), whereas that of the third cluster was not significantly different ($p = 0.277$).

No deaths occurred in the cocoon period in all these experiments, whereas we did not record the mortality of eggs and larvae before cocoon spinning.

Adult emergence under field conditions in summer generations

Of the cohort collected on 24 May 2007 (152 larvae spun cocoons from late May to late June), 99 adults emerged from early June to mid-August, 13 adults emerged around mid-

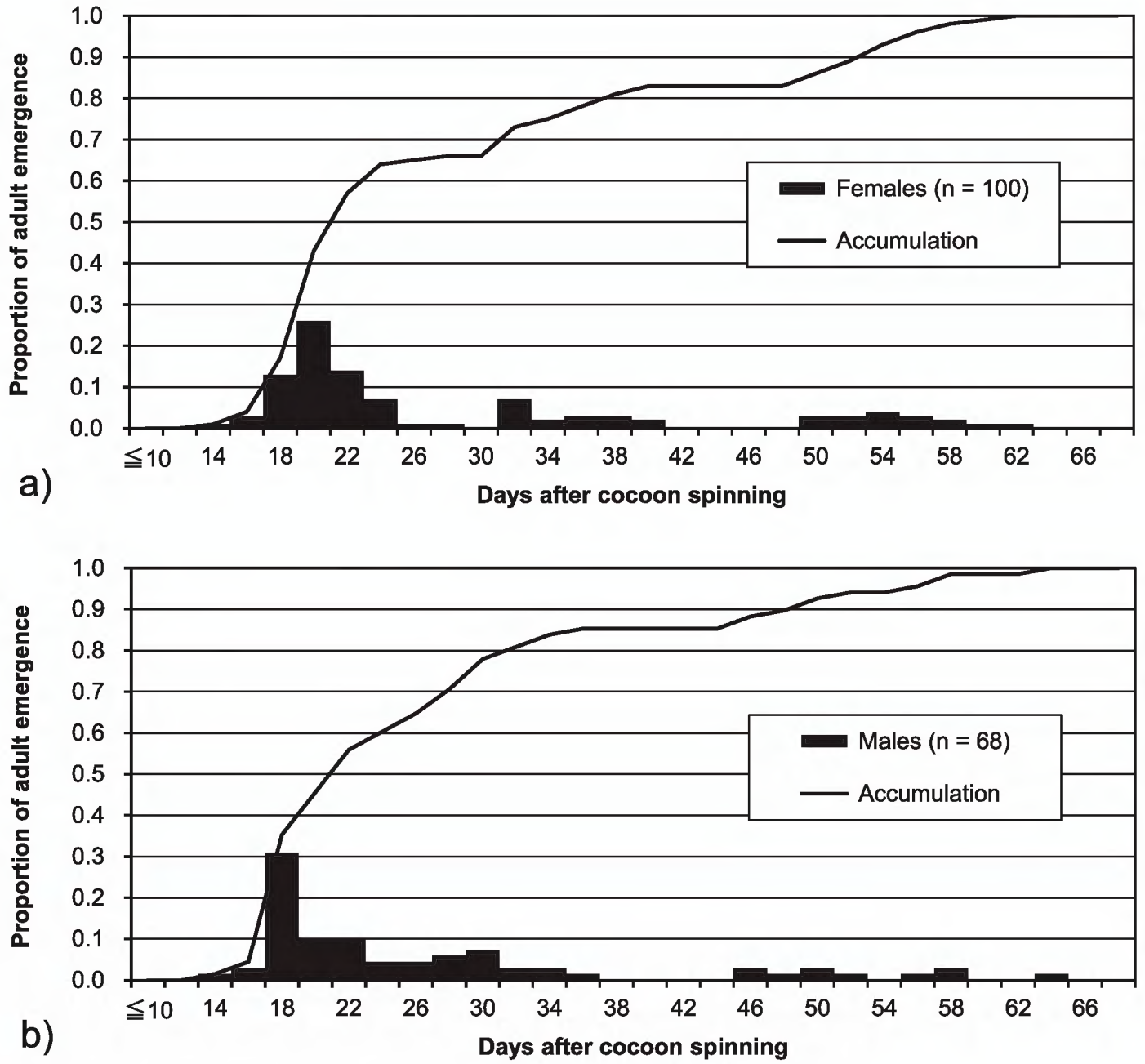

Figure 3. Adult emergence of females (**a**) and males (**b**) at 20°C with a 16L–8D photoperiod.

September (Fig. 6a), and the remaining individuals in cocoons died by the end of 2008, except for one male that emerged in early May 2008. Mortality in the cocoon period was 0.257 (39/152). A peak of adult emergence occurred around 26–30 days after cocoon spinning, following by a long tail of delayed emergence (Fig. 6b), and a cluster of adult emergence over 90 days after cocoon spinning was observed in September (cf. Fig. 6a).

Of the cohort collected on 4 July 2007 (93 larvae spun cocoons from mid- to late July), 38 adults emerged from early to mid-August, nine adults emerged around mid-September (Fig. 7a), and the remaining individuals in cocoons died by the end of 2008. Mortality in the cocoon period was relatively high, 0.495 (46/93). A peak of adult emergence was observed around 12–14 days after cocoon spinning with a short tail of delayed emergence (Fig. 7b), and the cluster of adult emergence over 40 days after cocoon spinning was in September (cf. Fig. 7a), which is the same as observed for the cohort collected in May.

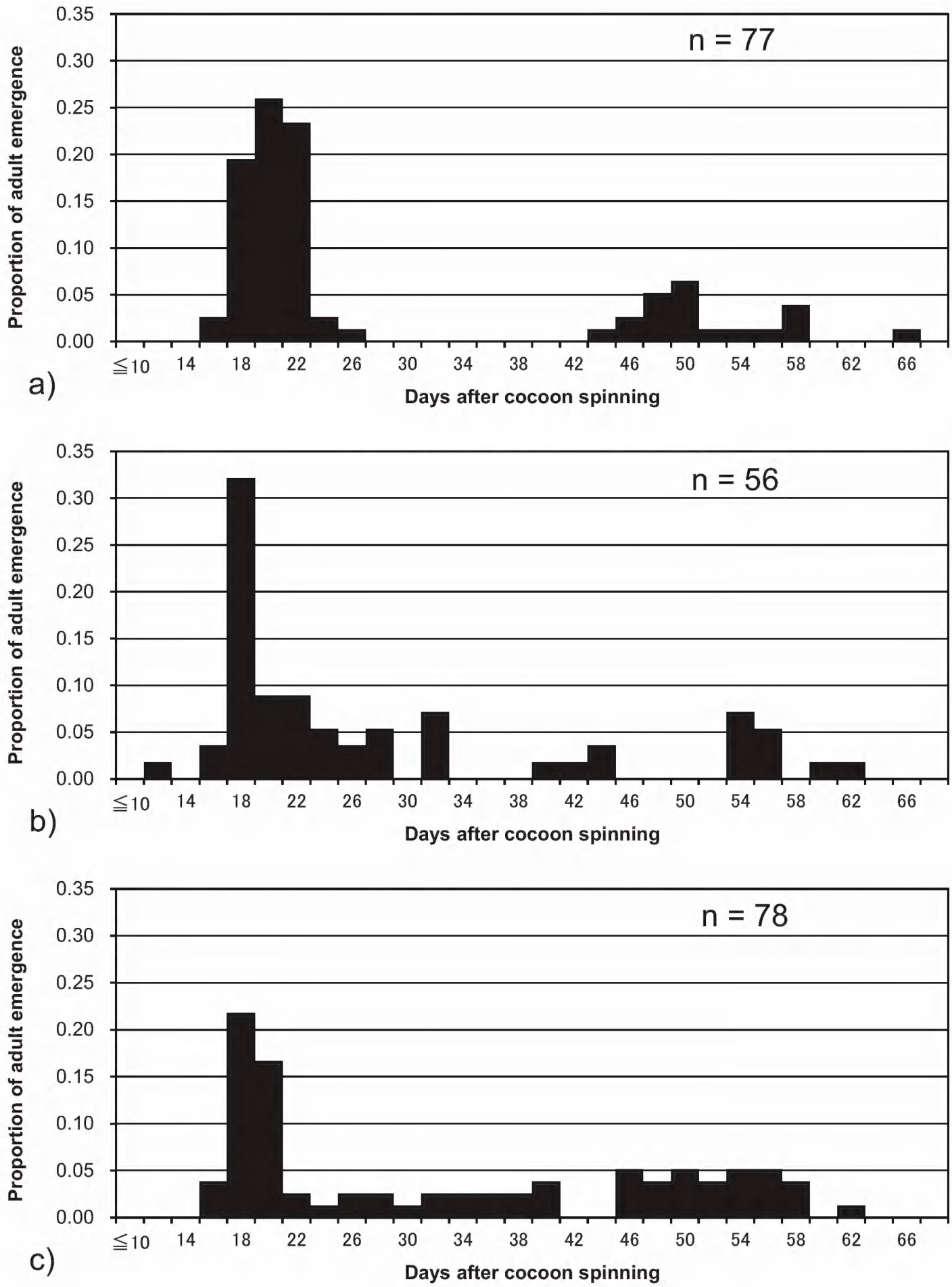

Figure 4. Adult emergence in the sons from mothers in the first (**a**), second (**b**), and third (**c**) clusters of emergence (see Fig. 3a) at 20°C with a 16L–8D photoperiod. The number of mothers in the first (**a**), second (**b**), and third (**c**) clusters of emergence was 16, 10, and 8 respectively.

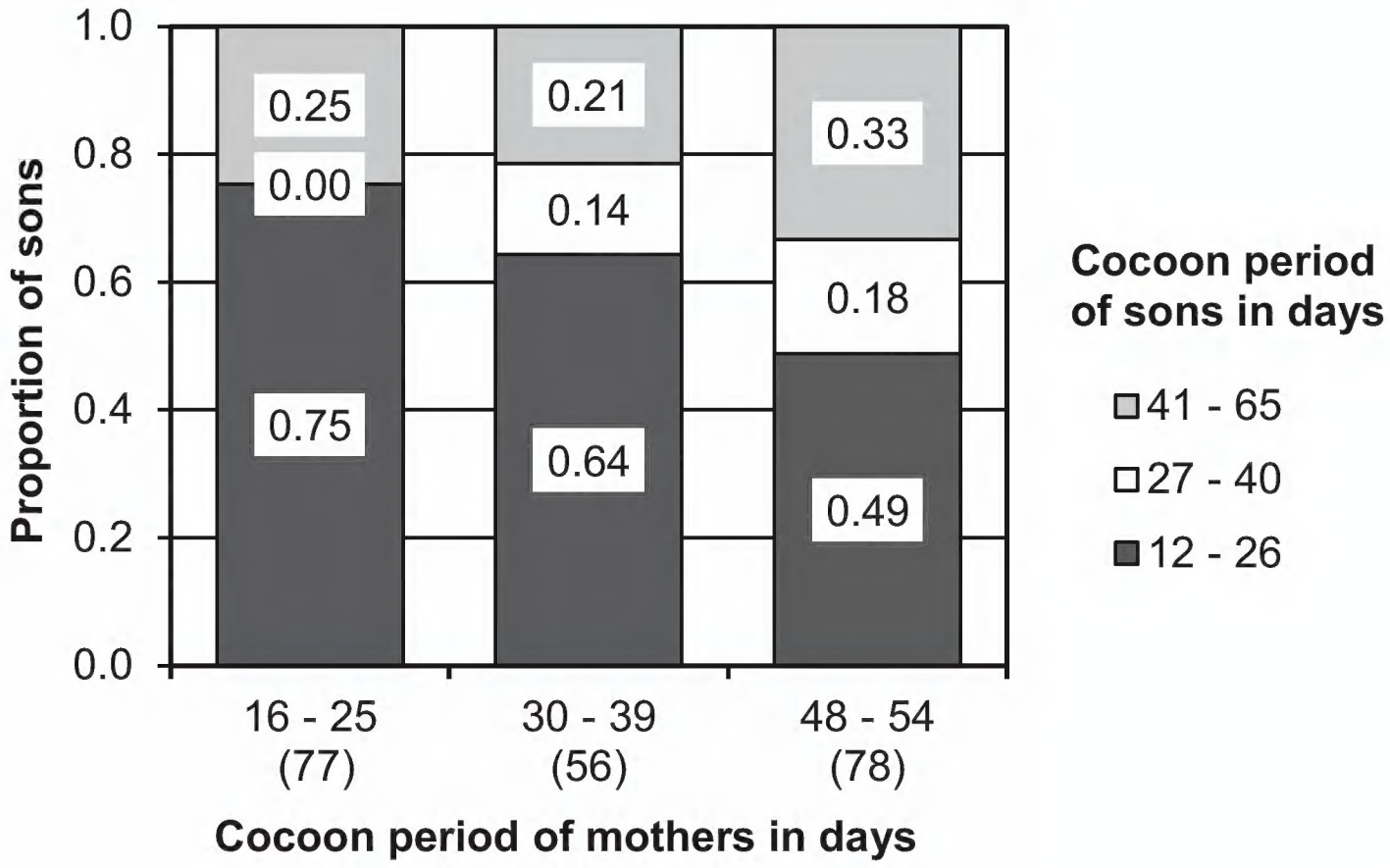

Figure 5. Proportion of the sons emerged after three cocoon periods for the mothers of three cocoon periods at 20°C with a 16L–8D photoperiod. Numbers in parentheses indicate the total numbers of sons that emerged.

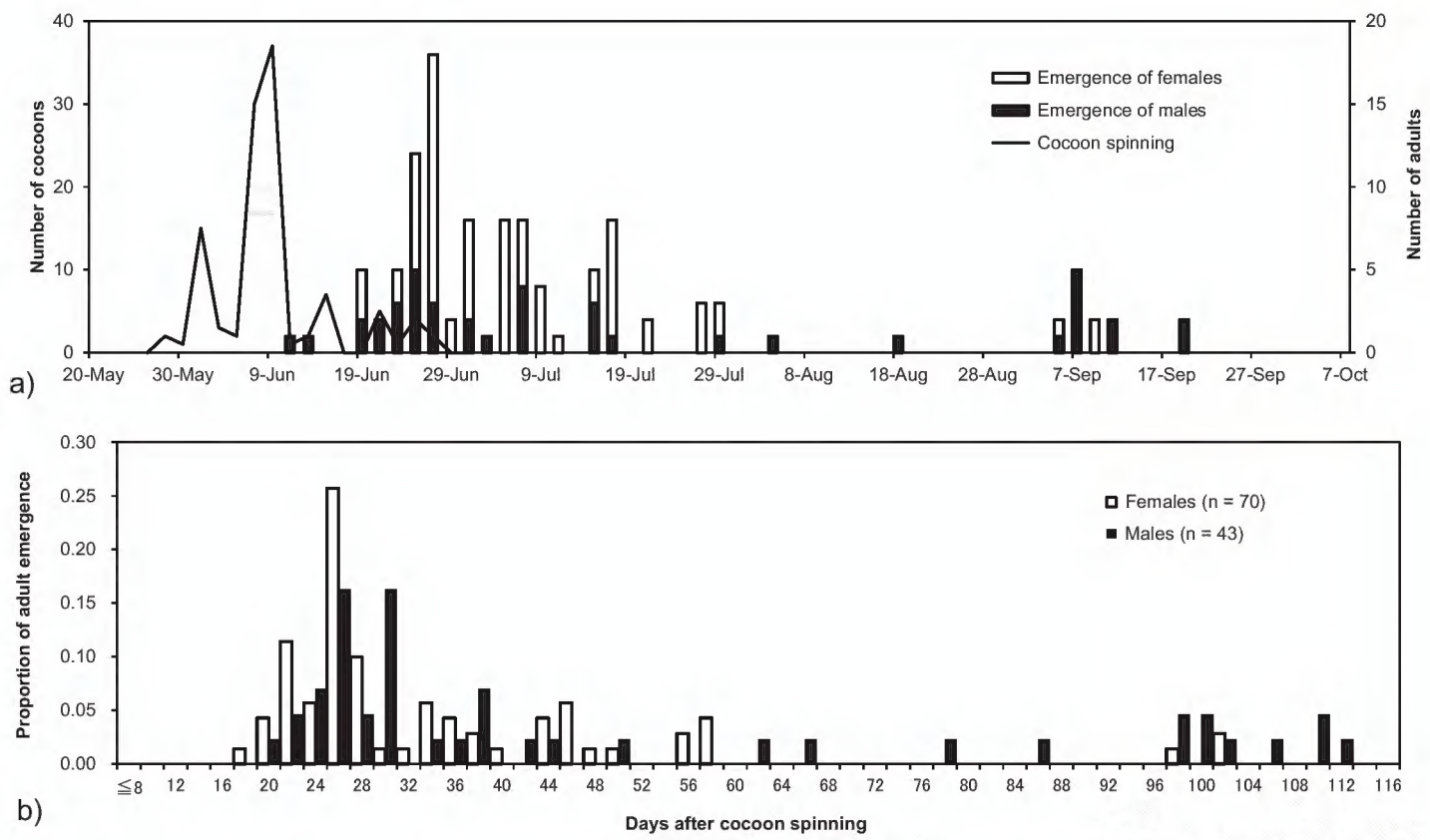

Figure 6. Cocoon spinning and adult emergence on the calendar (**a**) and the period from cocoon spinning to adult emergence (**b**) in the cohort collected on 24 May 2007.

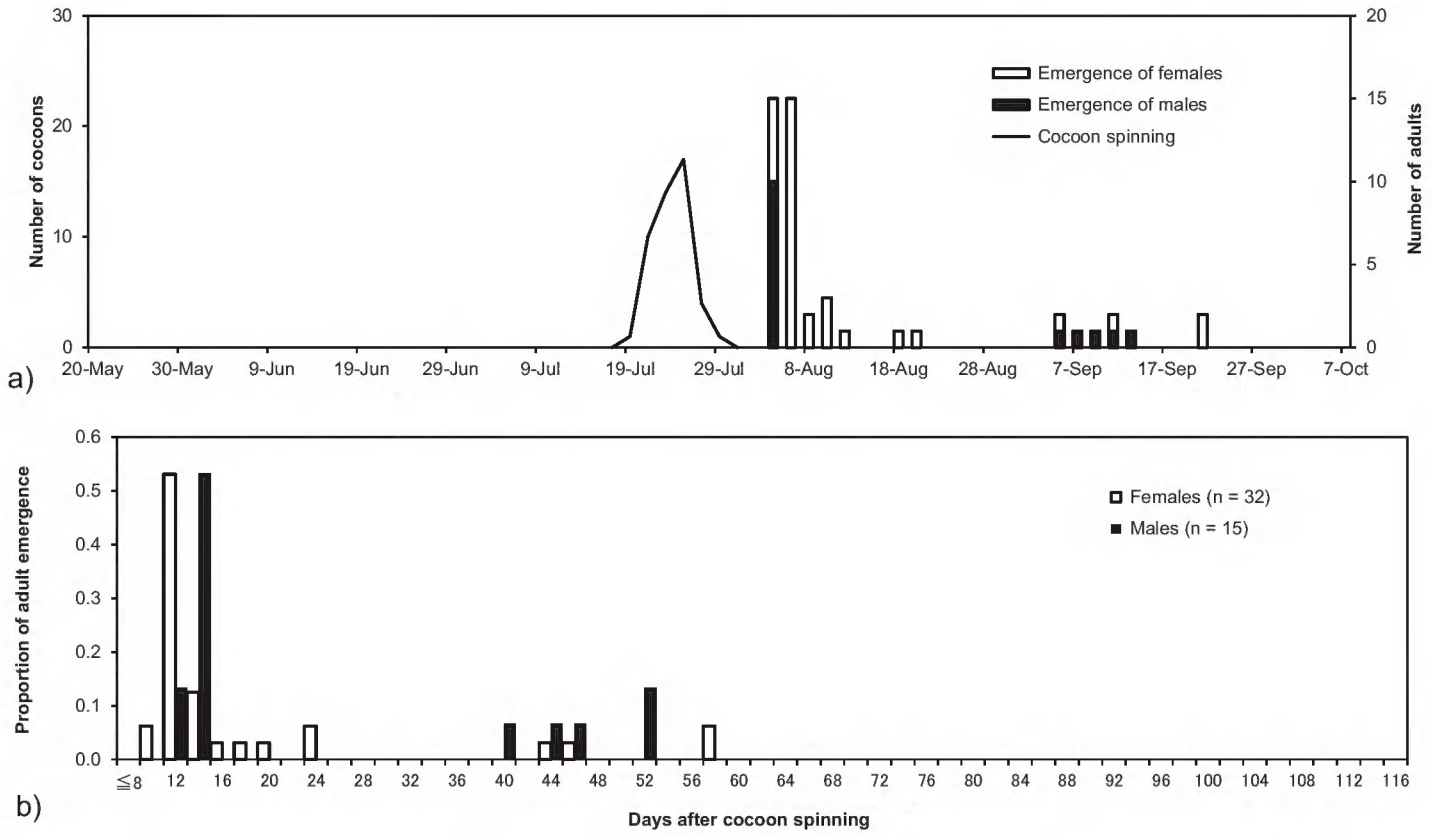

Figure 7. Cocoon spinning and adult emergence on the calendar (**a**) and the period from cocoon spinning to adult emergence (**b**) in the cohort collected on 4 July 2007.

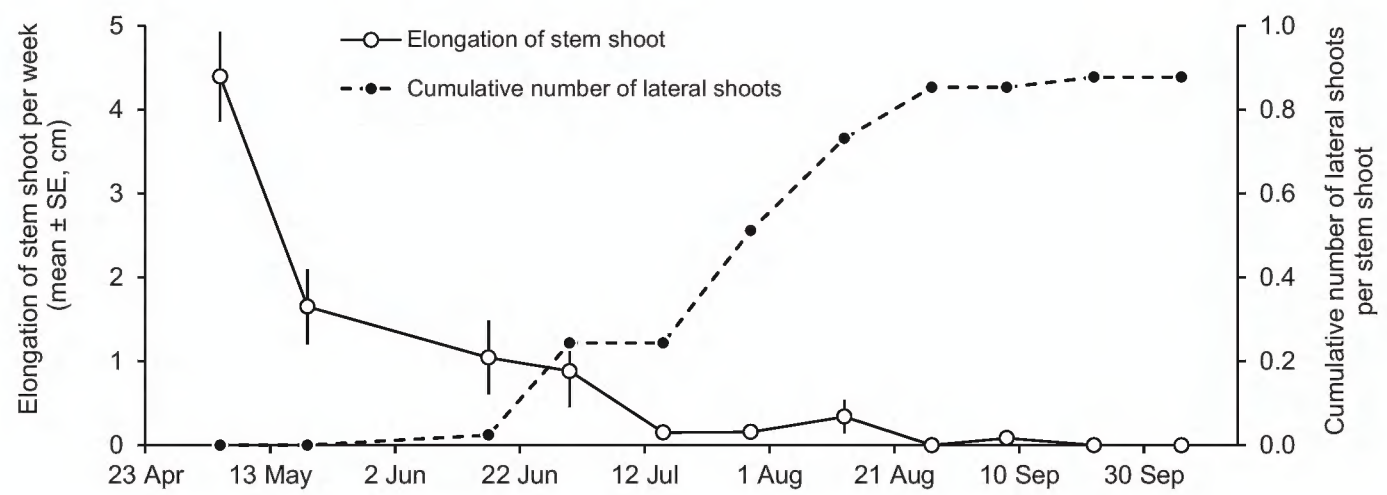

Figure 8. Elongation of the stem shoots of *Rosa multiflora*, along with the accumulation of its lateral shoots in 2007.

Shoot phenology of wild roses

Stem shoot elongation of wild roses started in late April and ceased in July when lateral shoots began to bud from the main shoots (Fig. 8). The accumulated number of the lateral shoots, which would elongate in fall, increased noticeably in July and August and leveled off in early September (Fig. 8).

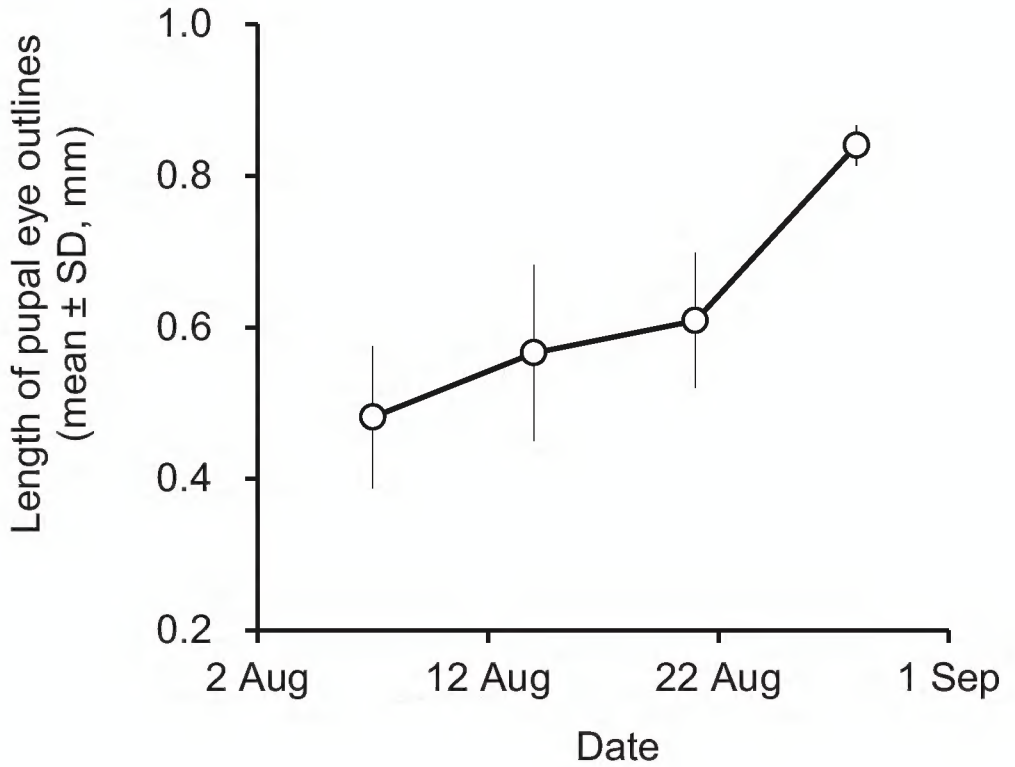

Figure 9. Changes in lengths of pupal eye outlines on prepupae in summer diapause under field conditions in 2008. Values are means ± SD (n = 8).

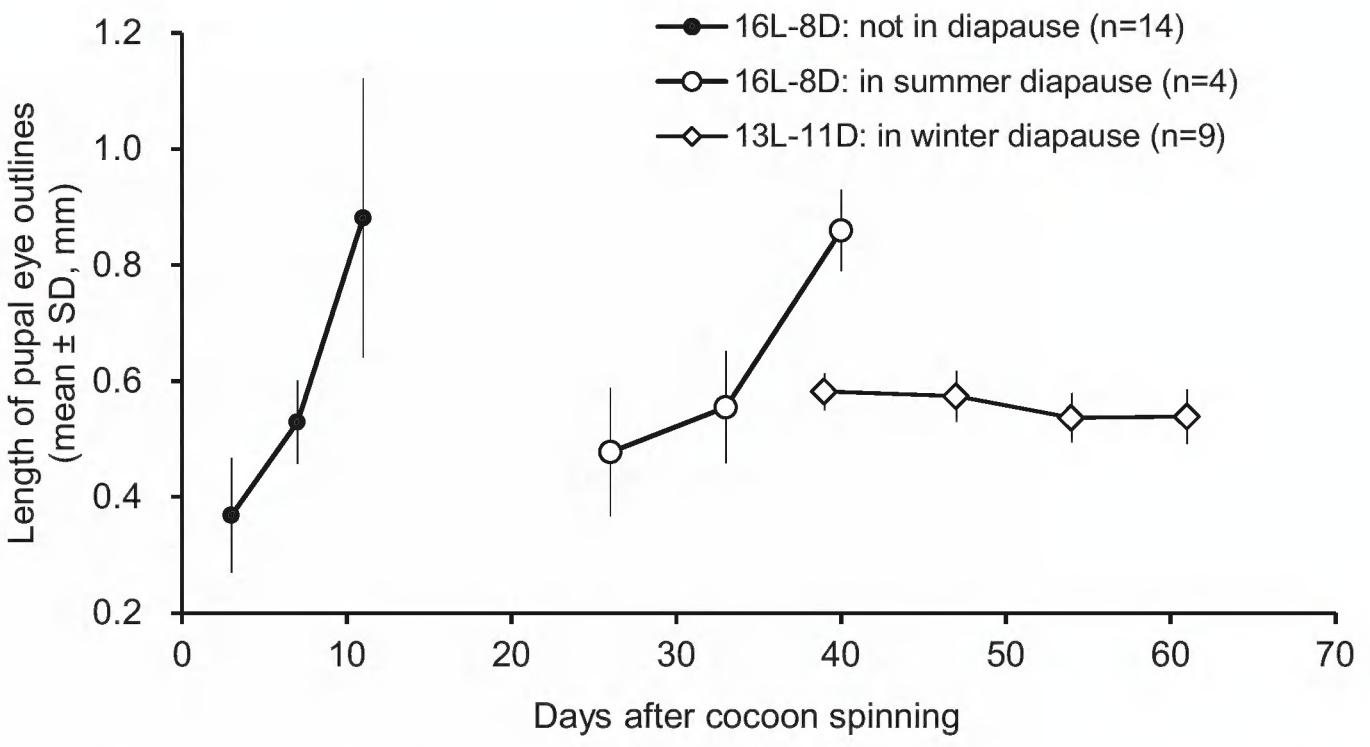

Figure 10. Changes in lengths of pupal eye outlines on prepupae not in diapause, in summer diapause at 20°C with a 16L–8D photoperiod, and in winter diapause at 20°C with a 13L–11D photoperiod. Values are means ± SD.

Prepupal development in cocoons

Under field conditions, the length of pupal eye outlines on the prepupae that remained over 40 days after cocoon spinning increased gradually until pupation in August (Fig. 9). At 20°C with the long-day (16L–8D) photoperiod, the pupal eye outlines of non-

diapause prepupae grew immediately after cocoon spinning, while those of prepupae in summer diapause increased gradually until pupation took place approximately 40 days after cocoon spinning (Fig. 10). At 20°C with the short-day (13L–11D) photoperiod, the length of pupal eye outlines of all prepupae remained short, likely reflecting winter diapauses (Fig. 10).

Discussion

Our laboratory experiments revealed that *Arge nigrinodosa* exhibits trimodal adult emergence under long-day conditions (Fig. 3). Soon after the first and largest cluster of emergence, a smaller second emergence takes place, which is followed by a third emergence after a discrete interval. Although the stage of delayed development to enable the second cluster of emergence was not identified, the third emergence was most likely caused by prepupal summer diapause (aestivation), in which the elongation of pupal eye outlines is temporarily interrupted at approximately the same size as in winter diapause (hibernation) under the short-day conditions (Fig. 10).

We analyzed the genetic background of polymodal adult emergence by comparing the emergence of sons from mothers of different adult emergences. The second cluster of emergence appears to be under genetic control because it was totally absent in the sons (haploid) of the mothers (diploid) in the first cluster of emergence (Fig. 5). In contrast, no heritability of the third cluster of emergence was supported given that its proportion was the same among the sons of mothers of all three clusters of emergence (Fig. 5).

The first cluster of adult emergence in the laboratory clearly corresponds to the early prevailing peak of adult emergence in the field, while the second one most likely represents a tail of delayed emergence after the peak (Figs 6, 7). The cluster of adult emergence occurred in September for both cohorts collected in May and July, and seems to match the third cluster of emergence in the laboratory. Synchronous adult emergence in September after prepupal summer diapause (Fig. 9) appears to be regulated by the changing day length and/or temperature in late summer (Baeza Larios and Ohno 2007), but why does it take place in September?

Given that *A. nigrinodosa* lays a large egg mass on a single rose shoot, the larvae often encounter food shortage (Adachi 1983b). Indeed, the food resources available to larvae drastically decline in summer, then increase again with the elongation of accumulated lateral shoots in fall (Fig. 8). Also, as shown in Figures 1 and 2, August is the most severe and highly variable month in terms of weather conditions. The extremely low rainfall in August, as observed during the last three decades, could be fatal to larvae, or at least severely worsen the food shortage. Therefore, partial summer diapause before adult emergence may be a risk-spreading (i.e., bet-hedging) strategy to cope with such uncertainty (Hopper 1999; Danks 2006). Alternative tactics for risk-spreading tend not to be inherited and are maintained as a stochastic polyphenism (Walker 1986) or adaptive coin-flipping (Cooper and Kaplan 1982). This is probably

the case for *A. nigrinodosa*, in which the third cluster of adult emergence is not heritable, but its proportion is genetically determined.

Conversely, the second cluster of adult emergence, which occurs soon after the first and normal emergence, appeared heritable, indicating genetic polymorphism. Furthermore, it probably takes place too soon after the normal emergence to disperse weather risks. Even though reliable data are lacking to gain a thorough understanding of the adaptive value of a slightly delayed adult emergence, note that a gregariously feeding sawfly such as *A. nigrinodosa* may incur a high risk of producing diploid males after consecutive sib-mating owing to a system of complementary sex determination (CSD) (Cook and Crozier 1995). While the sex determination mechanism of *A. nigrinodosa* has not been elucidated, according to Tikahiko Naito (personal communication), his unpublished data indicate CSD for the species. A slight delay of adult emergence after that of most siblings might be advantageous for reducing the production of sterile, diploid males.

The adult emergence of the rose sawfly *A. nigrinodosa* in summer generations appears to consist of three components as follows: the first emergence (i.e., the normal emergence); a second, slightly delayed genetically determined emergence; and a third emergence that takes place randomly after partial summer diapause. Thus, the concept of voltinism, the number of generations in a year, may not be applicable in the life history of sawflies with polymodal adult emergence, as indicated by Shinohara and Hara (2008). However, what is likely more important is the understanding that the apparently complicated life history of multivoltine sawflies is not random, but that it can be understood as a suite of adaptive tactics for dealing with a variety of environmental factors.

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